Quarterly Update

National Bioenergy Center Sugar Platform Integration Project

Biomass Program—Sustainable Fuels, Chemicals, Materials, and Power

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The Sugar Platform Integration Project focuses on integrating the processing steps involved in enzyme-based lignocellulose conversion technology. This project supports the U.S. Department of Energy's efforts to foster development, demonstration, and deployment of "sugar platform" biorefineries that produce inexpensive commodity sugars and fuel ethanol, as well as a variety of other fuel and chemical products, from abundant renewable lignocellulosic biomass.

The National Renewable Energy Laboratory manages this project for DOE's Office of the Biomass Program. Information on the Biomass Program is available at http://www.eere.energy.gov/biomass.html and information on Biofuels is posted at http://www.ott.doe.gov/biofuels/.

To discuss information in this update or for further information on the Sugar Platform Integration Project, contact Dan Schell at NREL, phone (303) 384-6869, email dan_schell@nrel.gov

NREL Participated in Corn Stover/Wheat Straw Harvesting Workshop, Imperial, NE, 2/4/04. Kim Magrini and Steve Thomas of NREL participated in a community workshop organized by Jim Hettenhaus (cea, Inc.) on the topic of corn stover and wheat straw harvesting in the town of Imperial, NE (Chase County). A report that Mr. Hettenhaus prepared for the Iogen Corporation identified Chase County as one of the top two locations (among 900 counties surveyed) in the U.S. for availability and harvestability of a year-round supply of biomass to use in a biomass conversion process. Speakers from Cargill-Dow, various USDA labs and NREL presented to an audience of 25-40 local farmers and ranchers.

R&D Progress

Effect of Scale up on Enzymatic Cellulose Hydrolysis. Differences in scale were not observed to effect enzymatic cellulose conversion in Amoco/NREL CRADA work conducted in the mid 1990s when simultaneous saccharification and cofermentation (SSCF) experiments were scaled up from the 2 L to the 9000 L fermentor scale. However, more recent experiments comparing the performance of enzymatic cellulose hydrolysis at the shake flask and bench fermentor scales have produced equivocal results indicating that there may be performance differences between shake flasks and fermentors. Since the assay routinely being used by the Enzymatic Hydrolysis Project's Enzyme Subcontract Liaison task to validate industry's enzyme performance improvements is based on a shake flask scale enzymatic cellulose hydrolysis assay, it is essential that we understand the source and magnitude of any performance differences occurring between different scales, and if necessary alter the standard assay methodology to make it more predictive of performance under anticipated processing conditions. To this end, we recently completed a series of comparative, replicated enzymatic cellulose saccharification experiments at the shake flask and bench fermentor scales. The extent of cellulose conversion achieved in duplicated bench fermentors was significantly lower than what was achieved in triplicated shake flasks. Several factors could produce this result, including shear inactivation of cellulases in agitated vessels and/or the inability to adequately mix the biomass slurry or control the temperature at high biomass solids concentrations in agitated bench-scale vessels. Work to further elucidate this phenomenon is underway. We believe that it will be possible to mitigate the impacts of performance differences between shake flasks and bench scale (and larger) agitated vessels through proper attention to reactor design, but further work is needed to demonstrate this, and we may need to incorporate novel design features better suited to mixing high solids slurries to do this.

Moving towards High Solids Saccharification. Progress in demonstrating high solids pretreatment indicate that substantial cost reduction in pretreatment equipment and operating cost should be possible. However, even further cost reductions can be realized if the high solid slurries produced by pretreatment can also be effectively saccharified at high solids concentrations. At the last Enzyme Sugar Platform Interim Stage Gate Review meeting (May 2003), the review panel suggested conducting this work. Achieving 20% or higher sugars concentrations in saccharification/fermentation from combined thermal/chemical hemicellulose hydrolysis and enzymatic cellulose hydrolysis will further reduce capital requirements. This task recently initiated a subcontracted effort to demonstrate the potential of a fed-batch feeding regime to achieve this goal. Preliminary saccharification work performed in agitated vessels using a simple feeding regime produced an approximate 20% increase in the solid loading when compared to batch operation at 17% solids. The ultimate goal is to achieve at least 25% solids loading in saccharification/fermentation and to use mathematical modeling to guide development of an effective feeding policy.

Improving Protein Measurement in Corn Stover. An overarching goal of this project is to improve methods for measuring biomass composition. Accurate accounting of biomass protein in agricultural feedstocks (corn stover) is important for closing mass balances around the various processing steps (e.g. pretreatment, saccharification), improving process modeling, and developing rapid compositional analysis methods. The crude protein content of biomass is calculated by multiplying the easily measured total nitrogen content of the material by a conversion factor. The conventional practice of using 6.25 as a nitrogen-to-protein conversion factor (N-factor) is based on an assumption that protein in biomass contains 16% nitrogen (100/16 = 6.25). We now know that this supposition is incorrect and we have been working to improve our approach by developing ways to calculate custom N-factors for common biomass feedstocks. This approach calculates the N-factor using data from a total nitrogen analysis and an amino acid analysis with multiple hydrolysis times. The crude protein measurement remains quick and inexpensive, but becomes more accurate once the custom N-factor is in place. Details of this method and examples of more accurate N-factors will be presented at the 26th Symposium on Biotechnology for Fuels and Chemicals in Chattanooga, TN.

Understanding Factors Affecting Corn Stover Compositional Variability. We are continuing to investigate the impact of corn stover compositional variability on bioconversion performance and process economics. Current efforts are focused on developing a better understanding of how genetic and environmental factors affect compositional variability. Understanding how to control feedstock variability is one of the keys to improving overall process economics. We have recently completed compositional analysis of corn stover samples collected from 9 fields located around Imperial (Chase County), NE using our existing near-infrared spectroscopy-based corn stover feedstock rapid analysis method. Preliminary results are very encouraging in that they show unexpectedly small variations in composition between the different samples. For example, the structural glucan content across all 9 fields averaged 34.5% (dry weight) with a standard deviation of only 1.3%. Likewise, xylan and lignin contents were 20.1% ± 1.3% and 16.8% ± 0.5%, respectively. These preliminary findings suggest that different varieties of corn stover farmed on different plots of land within the same limited locality using similar agronomic practices (including grain and stover harvesting methods) and subjected to the same weather pattern can yield compositionally similar corn stover. We are collecting more core samples from other fields near Imperial as baling operations continue to determine if this preliminary conclusion continues to hold over the length of the baling season for fields in this area (November - April). Additionally, a large quantity of corn stover from this region will be sent to NREL to support our research efforts and to supply external stakeholders.

Improving Methods for Measuring Biomass-Derived Sugars. The ability to produce high quality experimental data is constrained by the accuracy and precision with which sample chemical composition can be measured. Accurate measurements of sample compositions are the key to determining the amount of sugar that can be derived from a particular feedstock, as well as to quantifying conversion process efficiency and estimating overall process economics. Unfortunately, existing measurement techniques are derived from methods originally developed for woody feedstocks. As we have learned over the past couple of years, many of these existing methods are not well suited for analyzing herbaceous grassy feedstocks, like corn stover, which contain additional sugars and other components not present in wood. Significant efforts are being made to refine and develop more accurate compositional analysis methods for herbaceous feedstocks (and their process intermediates). Recently, Professor Foster Agblevor of Virginia Tech discovered a methodology that appears to significantly improve the accuracy of measuring mixed biomass sugars and their derivatives. Currently, a small subcontract is being placed with Professor Agblevor to determine if this new method is sufficiently reliable and accurate to become a new Biomass Program standard method. Results of this study will be communicated as they become available. Based on the limited information already available, we expect this new method to increase the accuracy and precision of measuring sugar (carbohydrate) levels in herbaceous feedstocks and related process intermediates.

Related Activities

Cellulase Subcontracts. Since mid-2000, the Biomass Program has been working with the two largest global enzyme producers, Genencor International and Novozymes Biotech Incorporated. The objective of this collaboration is to develop low-cost enzymes for biomass conversion. For the latest information, links to press releases provided by the enzyme producers are given below.

Novozymes reports 12-fold cost reduction

New Equipment Capabilities at NREL. Two new instruments, a Veeco Atomic Force Microscope (AMF) and a Near Field Optical Microscope, have been installed in temporary space at NREL. These instruments, along with a Transmission Electron Microscope and a Environmental Scanning Electron Microscope with delivery expected in mid summer, will be used to study biomass surface characteristics. The AFM has already been used to collect world-class images of the topography of cellulose in biomass.

Enzyme Sugar Platform Project Information. Web-based information on the ESP project including our recent presentations at stage gate review meetings can be found at the following link (ESP Project Information). A discussion of how Stage Gate management is used in the Biomass Program is also available at this site.

The 26th Symposium on Biotechnology for Fuels and Chemicals will be held May 9-12, 2004 at Chattanooga, TN. Various project team members will be presenting on protein measurements in corn stover, tracking the fate of calcium and sulfur in the “overliming” process for hydrolysate conditioning, and response surface modeling of high solids pretreatment.

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